

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Currently amended) An α -glucan phosphorylase having improved thermostability, which is obtained by modifying a natural α -glucan phosphorylase, wherein the natural α -glucan phosphorylase is ~~derived~~ obtained from a plant, and the α -glucan phosphorylase having improved thermostability has an amino acid residue which is different from that of the natural α -glucan phosphorylase and has an amino acid residue of C, I, L, V or W in at least one position selected from the group consisting of:

~~a position corresponding to position 4 in a motif sequence 1L: H-A-E-F-T-P-V-F-S or a position corresponding to position 4 in a motif sequence 1H: H-A-Q-Y-S-P-H-F-S;~~

~~a position corresponding to position 4 in a motif sequence 2: A-L-G-N-G-G-L-G; and]~~

~~a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V (SEQ ID NO: 47) or a position corresponding to position 7 in a motif sequence 3H: R-I-V-K-L-V-N-D-V];~~

wherein the α -glucan phosphorylase having improved thermostability has the same amino acid sequence as that of the natural α -glucan phosphorylase except for the substitution defined above, or has an amino acid sequence in which, in addition to substitution defined above, one or several amino acids are deleted, substituted or added relative to an amino acid sequence of natural α -glucan phosphorylase;

wherein the enzyme activity of the α -glucan phosphorylase having improved thermostability is equivalent or superior to the natural α -glucan phosphorylase; and

wherein enzyme activity of the α -glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the α -glucan phosphorylase having

improved thermostability at 37°C, before heating.

2. (Canceled)

3. (Currently amended) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein the amino acid sequence of the natural α -glucan phosphorylase has at least 50% identity with an amino acid sequence selected from the group consisting of: a from position 1 to position 916 of SEQ ID NO: 2; position 1 to position 912 of SEQ ID NO: 4; position 1 to position 893 of SEQ ID NO: 6; position 1 to position 939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID NO: 10; position 1 to position 971 of SEQ ID NO: 12; position 1 to position 983 of SEQ ID NO: 14; position 1 to position 928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID NO: 18; position 1 to position 832 of SEQ ID NO: 20; position 1 to position 840 of SEQ ID NO: 22; position 1 to position 841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID NO: 26; position 1 to position 841 of SEQ ID NO: 28; and a position 1 to position 838 of SEQ ID NO: 30].

4. (Currently amended) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein the amino acid sequence of the natural α -glucan phosphorylase is encoded by a nucleic acid molecule which hybridizes under stringent condition to a nucleic acid molecule consisting of a base sequence encoding an amino acid sequence selected from the group consisting of: a from position 1 to position 916 of SEQ ID NO: 2; position 1 to position 912 of SEQ ID NO: 4; position 1 to position 893 of SEQ ID NO: 6; position 1 to position 939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID NO: 10; position 1 to position 971 of SEQ ID NO: 12; position 1 to position 983 of SEQ ID NO: 14; position 1 to position 928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID NO: 18; position 1 to position 832 of SEQ ID NO: 20; position 1 to position 840 of SEQ ID NO: 22; position 1 to position 841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID NO: 26; position 1 to position 841 of SEQ ID NO: 28; and position 1 to position 838 of SEQ ID NO: 30,

wherein the stringent condition is a hybridization at 65°C in a solution containing

50% formamide, 5 xSSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5xDenhart's solution (0.2% BSA, 0.2% Ficoll 400 and 0.2% polyvinylpyrrolidone), 10% dextran sulfate, and 20 µg/ml denatured sheared salmon sperm DNA, and washing under the condition of 65°C using a SSC (saline-sodium citrate) solution having a 0.1 to 2-fold concentration, and

wherein the composition of the SSC solution having a 1-fold concentration is 150 mM sodium chloride and 15 mM sodium citrate.

5. (Currently amended) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein the natural α -glucan phosphorylase is a type L α -glucan phosphorylase, and the α -glucan phosphorylase having improved thermostability has an amino acid residue which is different from that of the natural α -glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1L; a position corresponding to position 4 in the motif sequence; 2 and a position corresponding to position 7 in the motif sequence 3L.

6. (Canceled)

7. (Currently amended) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid sequence of the natural α -glucan phosphorylase is selected from the group consisting of: position 1 to position 916 of SEQ ID NO: 2; position 1 to position 912 of SEQ ID NO: 4; position 1 to position 893 of SEQ ID NO: 6; position 1 to position 939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID NO: 10; position 1 to position 971 of SEQ ID NO: 12; position 1 to position 983 of SEQ ID NO: 14; position 1 to position 928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID NO: 18; position 1 to position 832 of SEQ ID NO: 20; position 1 to position 840 of SEQ ID NO: 22; position 1 to position 841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID NO: 26; position 1 to position 841 of SEQ ID NO: 28; and position 1 to position 838 of SEQ ID NO: 30.

8. (Currently amended) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein the natural α -glucan phosphorylase is derived obtained from potato or Arabidopsis thaliana.

Claims 9-15 (Canceled)

16. (Currently amended) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 7 in the motif sequence 3L or ~~a position corresponding to position 7 in the motif sequence 3H~~ is selected from the group consisting of C, I, L and V.

17. (Original) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein enzyme activity at 37°C of the α -glucan phosphorylase having improved thermostability after heated in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes is 30% or more of enzyme activity at 37°C of the α -glucan phosphorylase having improved thermostability, before the heating.

18. (Original) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein enzyme activity of the α -glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 65°C for 2 minutes, is 10% or more of enzyme activity of the α -glucan phosphorylase having improved thermostability at 37°C, before heating.

19. (Original) The α -glucan phosphorylase having improved thermostability according to claim 1, wherein storage stability thereof is improved as compared with the natural α -glucan phosphorylase.

Claims 20-33 (Canceled)

34. (Currently amended). An α -glucan phosphorylase having improved thermostability, which is obtained by modifying a plant-derived natural α -glucan phosphorylase obtained from a plant,

wherein the α -glucan phosphorylase having improved thermostability has an amino acid residue which is different from an amino acid residue of the natural α -glucan phosphorylase and has an amino acid residue C, I, L, V or W in at;

a position corresponding to position 4 in a motif sequence 1L: H-A-E-F-T-P-V-F-S or a position corresponding to position 4 in a motif sequence 1H: H-A-Q-Y-S-P-H-F-S;

a position corresponding to position 4 in a motif sequence 2L: A-L-G-N-G-G-L-G; and]

a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V (SEQ ID NO: 47) or a position corresponding to position 7 in a motif sequence 3H: R-I-V-K-L-V-N-D-V;

wherein the α -glucan phosphorylase having improved thermostability has the same amino acid sequence as that of the natural α -glucan phosphorylase except for the substitution defined above, or has an amino acid sequence in which, in addition to substitution defined above, one or several amino acids are deleted, substituted or added relative to an amino acid sequence of natural α -glucan phosphorylase,

wherein the enzyme activity of the α -glucan phosphorylase having improved thermostability is equivalent or superior to the natural α -glucan phosphorylase,

wherein the enzyme activity of the α -glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the α -glucan phosphorylase having improved thermostability at 37°C, before heating, and

wherein the α -glucan phosphorylase having improved thermostability has ability to synthesize an amylose having a weight average molecular weight of 600 kDa or more.

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Claims 35-40 (Canceled)